Design, Synthesis, and Evaluation of Carbamate-Substituted Analogues of (+)-Discodermolide

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ABSTRACT



The design, syntheses, and biological evaluation of 22 totally synthetic analogues of the potent microtubule-stabilizing agent (+)-discodermolide (1) have been achieved. Structure-activity relationships of the C(19) carbamate were defined, exploiting two synthetically simplified scaffolds, as well as the parent (+)-discodermolide framework.

In 1990, Gunasekera and co-workers reported the isolation and structural elucidation of (+)-discodermolide (**1**, Scheme 1) from the deep sea marine sponge *Discodermia dissoluta*.¹ Several years later (1996), Ter Haar and co-workers and Schreiber et al. independently revealed that (+)-discodermolide possesses potent antimitotic activity,² with a mechanism of action that, akin to the clinically proven anticancer agent Taxol (2), entails binding and stabilization of microtubules.² Importantly, (+)-discodermolide displays both significant tumor cell growth inhibitory activity against Taxol-resistant cells³ and cytotoxic synergy with Taxol in a variety of cell lines.⁴ Interest in (+)-discodermolide both as a synthetic target⁵ and a lead chemotherapeutic agent⁶ remains strong, as evidenced by five total syntheses^{Si-n} and ten papers devoted to analogues^{6g-p} published since 2001.

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In parallel with our efforts to develop an ever more practical (i.e., scalable) total synthesis of (+)-discodermolide

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to provide material for clinical development, we broadened our program to include the production of analogues designed to probe the structure—activity relationship, as well as to define the minimum critical structural element necessary for tumor cell growth inhibition.

Toward this end, we reported in 2001 that the simplified congeners (+)-14-normethyldiscodermolide (**3**, Scheme 2) and (+)-2,3-anhydrodiscodermolide (**4**) display cell growth



inhibitory activity rivaling that of the natural product.^{6f} Herein we describe the design, syntheses, and biological evaluation of derivatives of (+)-1, (+)-3, and (+)-4, which explore the influence of substitution at the C(19) carbamate.

Employing the synthetically simplified 14-normethyldiscodermolide scaffold for our initial investigations, we treated the previously disclosed secondary alcohol (+)-**5**^{6f} with a range of sterically and electronically varied isocyanates, followed by global deprotection to furnish the corresponding carbamates **6**–**16** in good to excellent yield (Scheme 3). Additionally, direct deprotection of (+)-**5** furnished descarbamoyl congener (+)-**17**.



In three of the four cell lines tested (Table 1), attachment of the phenyl, *p*-fluorophenyl, *p*-bromophenyl, or pyridyl substituents (**6**–**8**, **12**) led to a slight reduction in cell growth inhibition (a factor of 2–3), whereas carbamates possessing either a benzophenone moiety (**10**), electron-rich aromatics (**9**, **11**), or nonpolar substituents such as *n*-propyl, allyl, and benzyl (**13**–**15**), yielded analogues with potency comparable to that of the natural product. Alternatively, the phenyl sulfonamide congener **16** displays significantly reduced cytotoxicity, as does the carbamate-deletion analogue **17**.⁷

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Table 1. Cytotoxicity Observed for Analogues 6-17 cytotoxicity IC₅₀, nM MCF-7 NCI/ADR A549 SKOV-3 (+)-1 28 240 222146 8200 (+)-3 503548 >1000 135(+)-6 4742>1000 (+)-7 12547 (+)-8 84 >1000 24790 23>1000 23(+)-9 89 23>1000 67 36 (+)-10 6.22712 (+)-11 2000 27110 (+)-12 >100038 (+)-13 22 >1000 73 20 (+)-1417 4000 55 10 274000 30 (+)-1551 >1000 >1000 >1000 >1000 (+)-16

Also of significance, in marked contrast with (+)-discodermolide (1), which retains significant cell growth inhibitory activity in the Taxol-resistant *p*-glycoprotein-overexpressing cell line (NCI/ADR), (+)-14-normethyldiscodermolide and carbamoyl derivatives thereof generally demonstrate cytotoxicity only at concentrations greater than 1 μ M in this cell line.

>1000

>1000

(+)-17

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(+)-2,3-Anhydrodiscodermolide (4), isolated as a minor byproduct of global deprotection during our successful campaign to synthesize one gram of (+)-discodermolide, was found to be a more potent inhibitor of tumor cell growth than the natural product.^{6f} Thus, analogue (+)-4 and car-

 Table 2.
 Cytotoxicity Observed for Analogues 21–25

	cytotoxicity IC ₅₀ , nM				
	MCF-7	NCI/ADR	A549	SKOV-3	
(+)-1	28	240	22	21	
(+)-4	5.6	463	8.6	3.4	
(+) -21	5.6	260	9.4	7.6	
(+)- 22	120	>1000	400	250	
(+) -23	290	>1000	410	380	
(+) -24	630	>1000	2000	790	
(+) -25	660	>1000	2000	610	

bamoyl derivatives thereof were selected for synthesis and biological evaluation. Required for this venture was secondary alcohol (+)-19 (Scheme 4A), accessible via Wittig reaction of the previously described phosphonium salt (+)-AB^{5e} with the corresponding α,β -unsaturated lactone aldehyde (-)-18, followed by oxidative removal of the secondary PMB ether. Alternatively, (+)-19 can be obtained via basepromoted elimination of TBSOH from lactone (+)-20, again an advanced intermediate in our gram-scale synthesis of (+)discodermolide,^{5e} followed by DDQ-mediated removal of the PMB group. Aldehyde (-)-18, the requisite C(1)-C(8) fragment for our modular synthesis, was available in seven steps from known alcohol (-)-26⁸ (Scheme 4B).

In this series, the presence of the *N*,*N*-dimethylaniline moiety as in (+)-21 produced an analogue that was again superior in potency to the natural product (+)-1 in three of the four tested cell lines (Table 2). However, carbamates with pyridyl, benzyl, allyl, or propyl substituents [22–25], changes



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that had minimal effect on the cytotoxicity in the 14normethyl series (Table 1, **12–15**), severely degraded cell growth inhibitory activity when applied to the α , β -unsaturated lactone scaffold.

To extend the above carbamoyl substitutions to the natural (+)-discodermolide scaffold, the *n*-propyl, benzophenone, and *N*,*N*-dimethylaniline congeners **30–32** were prepared from the corresponding secondary alcohol (+)-**29**, a related late-stage intermediate in our synthesis of (+)-discodermolide^{5e} (Scheme 5). In addition, to probe further the effect of heteroatom incorporation in the carbamate moiety, imidazole (+)-**33** and morpholine (+)-**34** were also constructed and evaluated.



In this series, activity was again diminished relative to (+)-discodermolide (1) in the NCI/ADR drug-resistant cell line (Table 3). Conversely, in the three sensitive cell lines, the *n*-propyl-substituted analogue (+)-**30** proved to be only slightly less potent than (+)-discodermolide (1), while the benzophenone [(+)-**31**] and *N*,*N*-dimethylaniline [(+)-**32**] derivatives displayed a 3- to 14-fold increase in cell growth inhibitory activity. Introduction of alternate heteroatomic substructures as in (+)-**33** and (+)-**34** led to compounds that were generally equipotent with the natural product in the sensitive cell lines.

To summarize, 22 new, totally synthetic analogues of (+)-discodermolide (1) have been prepared and evaluated for

	cytotoxicity IC ₅₀ , nM				
	MCF-7	NCI/ADR	A549	SKOV-3	
(+) -1	28	240	22	21	
(+) -30	27	>1000	54	34	
(+) -31	8.4	>1000	10	8	
(+)- 32	1.9	450	5.1	1.8	
(+)-33	33	>1000	58	31	
(+)- 34	10	>1000	30	14	

tumor cell growth inhibitory activity in four human tumor cell lines. The NCI/ADR multidrug-resistant cell line appears to be very strict with respect to substitution at the carbamate, as only two analogues (21 and 32) retained submicromolar activity in this cell line. Potent cytotoxicity is, however, retained in the drug-sensitive cell lines when the carbamate is appended with a range of sterically and electronically varied substituents. In general, compounds derived from the parent (+)-discodermolide scaffold (1) and from the 14normethyl variant (3) were highly potent, while analogues stemming from (+)-2,3-anhydrodiscodermolide (4), which is itself more cytotoxic than the natural product, proved to be over an order of magnitude less active than (+)discodermolide. In conclusion, on the basis of the tolerance demonstrated by substitution at the C(19) carbamate, we believe that this region of the discodermolide skeleton holds considerable promise as a structural locale to alter favorably the pharmacokinetic profile of this potentially important class of chemotherapeutic agents.

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Supporting Information Available: Representative procedures, spectral data, and analytical data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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